ASSESSMENT OF FUNGAL PATHOGENS AS BIOCONTROL AGENTS OF *MYRIOPHYLLUM SPICATUM*

by

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Abstract

In the two years of this project(1994-95), nearly 200 sites in the UK and mainland Europe have been surveyed for fungal pathogens which could be used as biocontrol agents against *Myriophyllum spicatum.* Over 400 potential pathogens in 38 genera were obtained in pure culture. Isolates have been screened for pathogenicity on sections of plants, of these, 13 have been shown to possess some control capabilities. These include 2 isolates of *Gliocladium roseum,* 2 Indeterminate Hyphomycete (producing only chlamydospores), *Acremonium* sp, *Cylindrocarpon destructans, Embellisia* nr. *telluster, Fusarium solani, Geotrichum candidum Coniothyrium fuckelii, Cryptosporiopsis* sp, *Glomerella cingulata* and an Indeterminate Coelomycete.

Keywords

Myriophyllum spicatum, Biocontrol agents, fungal pathogens

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Introduction

Myriophyllum spicatum L. (or Eurasian watermilfoil) is a member of the Haloragidaceae family. It is a submerged aquatic plant which grows in a wide range of environmental conditions, in both fresh and brackish waters. In weedy situations, it is very fast growing, forming dense mats of foliage that interfere with the normal usage of water courses. Reproduction is by fragmentation of stems and the development of over-wintering buds; seed formation also occurs but may play little part in the spread of the weed.

M. spicatum is widely distributed throughout the U.K., with records from Cornwall through to the Outer Hebrides: and occurs in most European countries from Scandinavia in the North to Sicily in the South (Kew Herbarium Records). It also occurs in most of Asia as well as in East Africa (Harley & Forno, 1990). Although locally common throughout the natural range; it is rarely dominant and has never been reported as a weed problem. It is most frequently found in the U.K. in still water, especially in lime- rich areas. Other *Myriophyllum* spp. (i.e. *M. alterniflorum* and *M. proserpinacoides*) share its habitat, whilst *M. verticillatum* grows in faster- flowing water.

M. spicatum has been a problem in the United States since the 1930's (Harley and Forno, 1990). In the 1950's and 1960's it became a serious ecological and economical weed in larger bodies of water in North America. As an ecological problem, *M. spicatum* can greatly reduce the numbers of naturally occurring aquatic plant species, with records of a fall in species number from 20 to 9 in a two year period, with *M. spicatum* coverage increasing from 2% to 20-45% over the same period (Madsen, Sutherland, Bloomfield, Eichler & Boylen, 1990).

Attempts to control *M. spicatum* have involved both mechanical and chemical methods. Mechanical clearance can be cheaper than chemical alternatives, but needs to be carried out at least twice during the summer to produce a reasonable reduction in plant biomass. Herbicide applications have been successful, both underwater applications made by boat and aerial applications can give good control. However, because of environmental concerns applications of chemical herbicides need careful consideration. Due to the dilution from a body of water, large amounts of herbicide need to be applied, and, if control is not sufficient, reinfestation can be rapid. In addition, the chemical has to be specific, and be persistent enough to control the weed with no residual activity.

Many of the early investigations into biological control agents for *M. spicatum* concentrated on insects. Species on other *Myriophyllum* spp. from within the USA have been identified as possible control agents. A pyralid moth, *Acentria nivea,* found in stands of *Myriophyllum exalbescens* in the St Lawrence River caused leaf loss and girdling of stems (Batra, 1977). Surveys predominantly for insect agents have also been carried out in Pakistan, Bangladesh and much of Eastern Europe and Asia (Final report, CIBC Pakistan station 1965-1970; Harley &Forno, 1990). However, many of the insects found proved to be non-specific to the target weed and hence of limited use as biological control agents.

Use of pathogens has long been regarded as a good potential method of biological control for *M. spicatum* (Freeman &Charudattan, 1980). Work has been undertaken on isolating and assessing fungal pathogens from within the USA: *Acremonium curvulum* and *Fusarium sporotrichoides* were tested at Wisconsin, but, though capable of causing lesions, both failed to control the weed in large scale tests (Andrews &Hecht, 1981; Andrews, Hecht &Bashirian, 1982; Charudattan, 1990).

A fungal pathogen, *Colletotrichum gloeosporioides,* found on *M. spicatum* in Wisconsin, has been evaluated as a mycoherbicide, in combination with three possible chemical herbicides at down to 10% of their recommended concentration (Sorsa, Nordheim &Andrews, 1988). *Mycoleptodiscus terrestris,* from the southern States has also been tested against *M. spicatum* and a series of aquatic weeds and terrestrial crop plants, and has been shown to be virulent and reasonably specific (Verma & Charudattan 1993). Endophytic fungi have been reported in the literature on *Myriophyllum* sp., in both Europe and the USA (Sparrow, 1974; Luther, 1979) and appear to be very damaging.

M. spicatum constitutes part of the background or natural aquatic flora throughout most of Europe and rarely reaches weed status. However, some of these ecosystems (in Central Western Europe) have recently been invaded by the North American exotic species *Myriophyllum heterophyllum* (Spangehl & Scharrenberg, 1986). Domination by the latter species would indicate that a different spectrum of natural enemies occurs in Europe and that a search for a fungal biological control agent for *M. spicatum* within Europe would be beneficial.

Materials and Methods

Surveys

From plant records (Kew Herbarium, National Water Boards and the Terrestrial Ecological Surveys), sites of *M. spicatum* were selected to give a range of locations and environmental conditions. Sites were sampled over a two-year period (1994-1995) during the growing season (May-October). Both *M. spicatum* and other *Myriophyllum* species were collected, and samples of water and soil were also taken in some cases. Samples were taken back to the weed pathology laboratories of the International Institute of Biological Control (IIBC), Silwood Park, Ascot Berks (U.K.)

Isolations

Isolations from diseased tissues of *M. spicatum* collected during the surveys, were carried out following standard procedures, plants were washed under running tap water for two hours and rinsed in sterile distilled water before being placed on tap water agar (TWA). Samples of soil and water were also plated onto media selective for *Fusarium* (Komada, 1975), and specific baits were employed for Oomycetes and aquatic fungi. Cultures were forwarded to the International Mycological Institute (IMI), Egham, Surrey, (U.K.) for identification.

Screening

Isolates of species that are commonly pathogenic to plants, and those species that were isolated constantly from several sites were screened against *M. spicatum.*

Sections of plants (with two nodes) were cut, weighed (after excess surface water was removed), and placed in 100mls of sterile distilled water in a jar. These were inoculated with either two 9mm agar plugs or a 10^4 or 10^6 spores per ml suspension (dependent upon sporulation of the isolate) and kept at a constant 25°C with 12hr light, two uninoculated controls were included. After three weeks plants were visually assessed for any indication of infection. After a further two weeks samples were again visually assessed, reweighed (after excess surface water was removed), and plated onto TWA with antibiotics for identification and proof of pathogenicity (Koch's postulates). Comparison of initial and final weights was used to give an indication of an inhibitory effect in the absence of physical signs of infection (it was noted during field collecting, that plants generally show few lesions or other signs of infection).

Results and Discussion

Surveys

Over the two seasons of the project, surveys have been carried out at nearly two hundred sites in twelve European countries: covering most of England, Wales and Scotland, eastern France, northern Italy, northern Spain, northern Switzerland, southern Germany, central Austria, central Ireland, Portugal and Slovenia. Sites from which *M. spicatum* was collected have varied in character from ponds and drainage ditches to large lakes, rivers and canals. Plants were found in both still and fast flowing water, and at depths from 5-8 cm to 4-5 meters (in the clear waters of some of the southern European lakes). Though normally found in water of a neutral to alkaline pH, in a few sites in Scotland *M. spicatum* was found in water which, due to surrounding peat, was mildly acidic. As the acidity increased *M. spicatum* was replaced by *M. alterniflorium.*

Growth characteristics of the plants often varied, depending upon site features: in fast flowing, shallow rivers, plants had noticeably red stems which trailed up to 1 meter downstream and rooted at several points. In slower moving rivers and canals, plants had more branched stems, larger leaves (up to 3 cm in the Royal Canal, Ireland) and more surface detritus. In lakes, the major change in character was dependent upon the depth at which the plant was growing: at shallow edges, stems could be only a few centimetres long, increasing to several meters in deeper water. Plants grew deeper in the clearer and warmer southern European lakes compared to the more cloudy colder northern lakes in England and Scotland. When returned to the standard

laboratory conditions all plant samples grew in similar fashion indicating that these are ecotypes rather than biotypes.

Isolations

From the plant material (*M. spicatum* and related species), water and soil samples collected, over 400 isolates (from normally pathogenic genera) have been isolated, comprising 56 identified species in 39 genera (Appendix 2). There was no correlation between the species isolated and the collection site, either environmentally or geographically. The majority of isolates are common colonisers of plant tissues and genera such as; *Fusarium* and *Acremonium* have been routinely isolated from all types of locations. Significantly, *Gliocladium roseum* has only been isolated from lakes and ponds not from rivers. A few isolates are specific aquatic fungi; e.g. *Cylindrocarpon aquaticum* and *Nectria lugdunensis* from the Crinnean Canal in Scotland. Several isolates have been unusual records, such as the two *Embellisia* sp. isolated from Texel in Holland and Slapton Ley in England which had only previously been recorded from desert soils in Wyoming *Sclerotium hydrophilum*, isolated from Afrilzer See in Austria has previously been recorded on *M. spicatum* in Yugoslavia (IMI Culture collection).

Screening

In total, 291 isolates have been tested, of these 15 have shown some degree of pathogenicity or control, causing a reduction in growth (assessed by weight) and in more severe cases, loss of leaves, necrosis or death (Table 2). The majority of isolates damage the older tissue of the plant and have only a minimum affect on the newer growth. Of the isolates giving some degree of control, 12 of these were reisolated from the plant tissue.

Of these twelve isolates which satisfy Koch's postulates, two are still unidentified Hyphomycetes (Mir 49a and Mir 80c) and two Coelomycetes (Mir 35 and Mir 36). Identification has been hampered by their very low and sporadic sporulation, though this does not appear to hinder either infection or reisolation from plant tissues.

Three of the isolates showing some degree of control are similar to those already screened in the USA (Andrews & Hecht 1981; Andrews *et al* 1982; Charudattan 1990;Verma & Charudattan 1993). *Acremonium* sp. (Mir 68c) has been screened twice giving good results and was reisolated both times. Results of reisolation of *Fusarium sporotrichoides* (Mir 96b) are still pending, but the isolate has been able to cause the death of inoculated plant sections. The native American isolates screened; *Acremonium curvulum* and *Fusarium sporotrichoides* (Andrews & Hecht 1981; Andrews *et al* 1982; Charudattan 1990), were successful in small scale tests, but failed to control the weed in large trials. Though this may be the case with the European isolates, their closer evolution with the plants should allow for more consistent results.

An isolate of *Colletotrichum gloeosporioides* (teleomorph: *Glomerella cingulata*), has been tested as a mycoherbicide in the USA (Sorsa *et al* 1988), whilst the European strain (Mir 51), though not reisolated from plant tissue, has been screened twice, and reduced growth rate in both tests. Significantly, *Mycoleptodiscus terrestris*,which has been isolated in both the USA and China, and shown to be virulent and reasonably specific to *M. spicatum* (Verma & Charudattan 1993), was not found during any of the European surveys.

Several of the isolates which have shown a degree of control (*Cylindrocarpon destructans, Fusarium solani, Coniothyrium fuckelii, Geotrichum candidum* and *Gliocladium roseum*) are generally not regarded as pathogenic or specific, their ability to infect *M. spicatum* was probably opportunistic, aided by the small plant sections used in the screen, and may not be repeatable with whole plants.

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Appendices

Appendix 1

Locations of collection sites

CODE	DATE	SITE
MIR 1-9	04/09/93	Slapton Ley, Slapton, Devon, ENGLAND
MIR 10-12	05/09/93	Cherrybrook, High Dartmoor, Devon, ENGLAND
MIR 13	12/10/93	Streets Heath Pond, Chobham, Surrey, ENGLAND
MIR 14	12/10/93	Wood Street Pond, Guilford, Surrey, ENGLAND
MIR 15	posted	Southern Prague Lakes, CZECH REPUBLIC
MIR 16	03/11/93	Wicken Fen Canal, Cambridgeshire, ENGLAND
MIR 17	06/11/93	South Ferring Pond, Worthing, Sussex, ENGLAND
MIR 18	28/08/93	Angermünde, GERMANY
MIR 19	01/09/93	Großer Buckowsee, Eberswalde, GERMANY
MIR 20	03/09/93	Oder-Havel-Kanal, Eberswalde, GERMANY
MIR 21	27/07/93	Marzurskie, POLAND
MIR 22	posted	Heider See, Bonn, GERMANY
MIR 23	02/01/94	De Koog Pond, Texel Island, NEATHERLANDS
MIR 24	12/05/94	Wood Street Pond, Guilford, Surrey, ENGLAND
MIR 25	25/05/94	Pett Pond, Winchelsea, Sussex, ENGLAND
MIR 26	25/05/94	Drainage Ditch, Winchelsea, Sussex, ENGLAND
MIR 27	25/05/94	St Michaels on Wyre, Fleetwood, Lancashire, ENGLAND
MIR 28-31	09/06/94	Roe Ponds, Hardwick Hall, Derbyshire, ENGLAND
MIR 32	09/06/94	Feeder Pond, Chatsworth Palace, Derbyshire, ENGLAND
MIR 33	20/06/94	Finger Pond, Priory Park, Bedfordshire, ENGLAND
MIR 34	20/06/94	Harold and Odell Lake, Harold, Bedfordshire, ENGLAND
MIR 35	20/06/94	River Great Ouze, Harold and Odell Country Park, Bedfordshire, ENGLAND
MIR 36	21/06/94	Soham Lode, Soham, Cambridgeshire, ENGLAND
MIR 37	28/06/94	River Axe, Axminster, Devon, ENGLAND
MIR 38	28/06/94	River Axe, Colyford, Devon, ENGLAND
MIR 39	28/06/94	Exeter Canal, Countess Weir, Devon, ENGLAND
MIR 40	28/06/94	Exeter Canal, Topsham Lock, Exminster Marshes, Devon, ENGLAND
MIR 41	29/04/94	Slapton Ley, Slapton, Devon, ENGLAND
MIR 42	20/04/94	River Frome, Moreton, Dorset, ENGLAND
MIR 43	29/04/94	River Piddle, Wool Bridge, Dorset, ENGLAND
MIR 44	05/07/94	Pit 16, Cotswold Water Park, Gloucestershire, ENGLAND
MIR 45	05/07/94	Lake 31, Keynes Country Park, Gloucestershire, ENGLAND
MIR 46	05/07/94	Lake 32, Keynes Country Park, Gloucestershire, ENGLAND
MIR 47	05/07/94	Lake 56, Neighbridge Country Park, Gloucestershire, ENGLAND
MIR 48	05/07/94	Lake Below 56, Neighbridge Country Park, Gloucestershire, ENGLAND
MIR 49	06/07/94	River Hart, Bramshill, Hampshire, ENGLAND
MIR 50	06/07/94	Whitewater River, North Warnborough, Hampshire, ENGLAND
MIR 51	06/07/94	Basingstoke Canal, Broad Oak Bridge, Hampshire, ENGLAND
MIR 52	06/07/94	Basingstoke Canal, Dogmersfield, Hampshire, ENGLAND
MIR 53	16/07/94	River Great Ouze, Clapham, Bedfordshire, ENGLAND
MIR 54	19/07/94	River Wylye, Great Wishford, Wiltshire, ENGLAND
MIR 55	19/07/94	River Nadder, Compton Chamberlayne, Wiltshire, ENGLAND
MIR 56	19/07/94	River Stour, Childe Okeford, Wiltshire, ENGLAND
MIR 57	19/07/94	River Stour, Dudsbury Golf Course, West Parley, Dorset, ENGLAND
MIR 58	20/07/94	River Avon, Kingston, Dorset, ENGLAND
MIR 59	20/07/94	Dockens Water, Rockford, Hampshire, ENGLAND
MIR 60	20/07/94	Funtley Lake, Funtley, Hampshire, ENGLAND
MIR 61	28/07/94	Burton Mere Trout Pond, Burton, South Wirral, Cheshire, ENGLAND
MIR 62	04/08/94	Kenfig Pond, South Glamorgan, WALES

CODE	DATE	SITE
MIR 63	04/08/94	Llangorse Lake, Powys, WALES
MIR 64	04/08/94	Llan Bwch-llyn Lake, Powys, WALES
MIR 65	05/08/94	Broxwood Court Pond, Broxwood, Hereford and Worcestershire, ENGLAND
MIR 66	05/08/94	River Arrow, Ivington, Hereford and Worcestershire, ENGLAND
MIR 67	17/08/94	Needham Market Fishing Lake, Needham Market, Suffolk, ENGLAND
MIR 68	18/08/94	Chantry Point Ditch, Orford, Suffolk, ENGLAND
MIR 69	02/09/94	Llangorse Lake, Powys, WALES
MIR 70	05/09/94	River Eamont, Ullswater, Cumbria, ENGLAND
MIR 71	05/09/94	Derwent Water, North of Derwent Isle, Cumbria, ENGLAND
MIR 72	05/09/94	Derwent Water, East of Lords Isle, Cumbria, ENGLAND
MIR 73	06/09/94	Buttermere, Cumbria, ENGLAND
MIR 74	06/09/94	Crummock Water, Cumbria, ENGLAND
MIR 75	06/09/94	Ennerdale Water, Cumbria, ENGLAND
MIR 76	06/09/94	Loweswater, Cumbria, ENGLAND
MIR 77	07/09/94	River Irt, Wastwater, Cumbria, ENGLAND
MIR 78	07/09/94	Nether Beck, Wastwater, Cumbria, ENGLAND
MIR 79	07/09/94	Conniston Water, Cumbria, ENGLAND
MIR 80	07/09/94	Grasmere, Cumbria, ENGLAND
MIR 81	07/09/94	Rydal Water, Cumbria, ENGLAND
MIR 82-83	17/09/94	Lough Ree, River Shannon, Barley Harbour, IRELAND
MIR 84	17/09/94	Royal Canal, Ballynacargy, IRELAND
MIR 85	18/09/94	The Grand Canal, Edenderry, IRELAND
MIR 86	18/09/94	Lough Derravaragh, Castle Pollard, IRELAND
MIR 87	18/09/94	The Grand Canal, Rathangas, IRELAND
MIR 88	18/09/94	River Slate, Rathangas, IRELAND
MIR 89	18/09/94	River Liffey, Clane, IRELAND
MIR 90	03/10/94	Lake Geneva, Nyon, SWITZERLAND
MIR 91	04/10/94	Lake Neuchâtel, Colombie, SWITZERLAND
MIR 92	04/10/94	Etang de Maissausis, La Chapelle sous Chaux, FRANCE
MIR 93	05/10/94	Lac de Longmer, Langmer, Gerardmer, FRANCE
MIR 94	05/10/94	Stream north of Schaenau, Rhinau, FRANCE
MIR 95	05/10/94	River Rhine, Rhinau, FRANCE
MIR 96	05/10/94	Canal du Rhône au Rhin, Neunkirch, FRANCE
MIR 97	05/10/94	Etang de Stock, Diane-et-Kerpick, Gorraie, FRANCE
MIR 98	06/10/94	River Moselle, Trieir, GERMANY
MIR 99	07/10/94	Feilinger See, west of Koblenz, GERMANY
MIR 100	29/04/95	Loch Ness Centre Pond, Drummandrochit, Highland Region, SCOTLAND
MIR 101	30/04/95	Pond off River Moristin, Glen Moriston, Highland Region, SCOTLAND
MIR 102	30/04/95	River Moristin, Glen Moriston, Highland Region, SCOTLAND
MIR 103	30/04/95	River Schiel, near Loch Duich, Highland Region, SCOTLAND
MIR 104	01/05/95	Tarn off Road, Kilmalaug, Isle of Skye, Highland Region, SCOTLAND
MIR 105	01/05/95	Tarn at Staffin, north Skye, Isle of Skye, Highland Region, SCOTLAND
MIR 106	02/05/95	River Schnizort, Dunvegen, Isle of Skye, Highland Region, SCOTLAND
MIR 107	02/05/95	River at Bernisdale, Isle of Skye, Highland Region, SCOTLAND
MIR 108	02/05/95	River Drynock, Carbost, Isle of Skye, Highland Region, SCOTLAND
MIR 109	03/05/95	River at Pentland Road, Isle of Lewis, Highland Region, SCOTLAND
MIR 110	03/05/95	River Greeta, Pentland, Isle of Lewis, Highland Region, SCOTLAND
MIR 111	03/05/95	River at Chanais, Boderer, Isle of Lewis, Highland Region, SCOTLAND
MIR 112	03/05/95	River at Leiniscal, Isle of Lewis, Highland Region, SCOTLAND
MIR 113	03/05/95	River to Loch Lathainuel, Isle of Lewis, Highland Region, SCOTLAND
MIR 114	04/05/95	River at Bahii Allen, Isle of Lewis, Highland Region, SCOTLAND
MIR 115	04/05/95	River at Loch mouth, Tarbet, Isle of Lewis, Highland Region, SCOTLAND
MIR 116	04/05/95	River at Tarbet, Isle of Lewis, Highland Region, SCOTLAND
MIR 117	05/05/95	River at Strathkaiard, Ullapool, Highland Region, SCOTLAND
MIR 118	05/05/95	Loch at Knockau, Ullapool, Highland Region, SCOTLAND
MIR 119	05/05/95	River at Benmore, Ledmore Junction, Highland Region, SCOTLAND

CODE	DATE	SITE
MIR 120	05/05/95	River Oakley, Ledmore, Highland Region, SCOTLAND
MIR 121	23/05/95	Cleethorpes Country Park Lake, Cleethorpes, Humberside, ENGLAND
MIR 122	23/05/95	Louth Canal, Tetney Lock, south of Cleethorpes, Lincolnshire, ENGLAND
MIR 123	23/05/95	Trout Pond (1), Maltby le Marsh, Mabelthorpe, Lincolnshire, ENGLAND
MIR 124	23/05/95	River at Yarburgh, Louth, Lincolnshire, ENGLAND
MIR 125	23/05/95	Fishing Pond (1), Maltby le Marsh, Mabelthorpe, Lincolnshire, ENGLAND
MIR 126	24/05/95	River Bain, Coningsby, Sleaford, Lincolnshire, ENGLAND
MIR 127	23/05/95	Fishing Pond (2), Maltby le Marsh, Mabelthorpe, Lincolnshire, ENGLAND
MIR 128	22/05/95	Lakes at Ealand, Humberside, ENGLAND
MIR 129	23/05/95	Trout Pond (2), Maltby le Marsh, Mabelthorpe, Lincolnshire, ENGLAND
MIR 130	06/06/95	Rookley Lake, Rookley Country Park, Isle of Wight, ENGLAND
MIR 131	07/06/95	Alvington Manor Pool, Carisbrooke, Isle of Wight, ENGLAND
MIR 132	12/06/95	Bala Lake, Gwynedd, WALES
MIR 133	13/06/95	River Teme, Hereford and Worcestershire, ENGLAND
MIR 134-135	13/06/95	River at Oversley Green, Alcester, Hereford and Worcestershire, ENGLAND
MIR 136	04/07/95	Lago di Maggiore, Bareno, Arona, Peidmont, ITALY
MIR 137	04/07/95	Lago di Monate, Monate, Lombardi, ITALY
MIR 138	04/07/95	Lago di Varese, Biandronno, Lombardi, ITALY
MIR 139	04/07/95	Lago di Como, Cernobbio, Lombardi, ITALY
MIR 140	05/07/95	Lago di Endine, Sponone al Lago, Lombardi, ITALY
MIR 141	05/07/95	Lago d'Idro, opposite Idro, Lombardi, ITALY
MIR 142	06/07/95	Lago di Garda, Maderno, Lombardi, ITALY
MIR 143	06/07/95	River Site, Quarto d'Altino, Veneto, ITALY
MIR 144	06/07/95	River at Oderzo, Veneto, ITALY
MIR 145	06/07/95	River at Blessaglia, Veneto, ITALY
MIR 146	06/07/95	River at Pordenone->Udine Road, Veneto, ITALY
MIR 147	07/07/95	Lake Bohinjskajez, Ribcev Laz, SLOVENIA
MIR 148	07/07/95	Lake Bled, Bled, SLOVENIA
MIR 149	08/07/95	Afrilzer See, north of Villach, AUSTRIA
MIR 150	08/07/95	Brennsee, north of Villach, AUSTRIA
MIR 151	08/07/95	Millstater See, Spittal, AUSTRIA
MIR 152	08/07/95	Mondsee, east of Salzburg, AUSTRIA
MIR 153	08/07/95	Attersee, east of Salzburg, AUSTRIA
MIR 154	07/08/95	Lochgelly, north east of Dumferlin, Fife, SCOTLAND
MIR 155	07/08/95	Loch Ore, Ballingry, Fife, SCOTLAND
MIR 156	07/08/95	River Tay, Perth Racecourse, Tayside, SCOTLAND
MIR 157	08/08/95	River South Esk, Brechin-> Forfar, Tayside, SCOTLAND
MIR 158	08/08/95	River Don, Inverurie, Grampian Region, SCOTLAND
MIR 159	08/08/95	River Ythan, Methlick, Grampian Region, SCOTLAND
MIR 160	08/08/95	River Deveron, Turriff, Grampian Region, SCOTLAND
MIR 161	09/08/95	Loch Morlich, east of Aviemore, Highland Region, SCOTLAND
MIR 162	09/08/95	Loch an Eilein, south of Aviemore, Highland Region, SCOTLAND
MIR 163	09/08/95	Loch Insh, south west of Aviemore, Highland Region, SCOTLAND
MIR 164	09/08/95	Loch Tay, Kenmore, Tayside, SCOTLAND
MIR 165	09/08/95	Loch on River Dochen, Benmore, Central Region, SCOTLAND
MIR 166	10/08/95	Loch Lomond, Inveriglas, Strathclyde, SCOTLAND
MIR 167	10/08/95	Loch above Loch Long, Strathclyde, SCOTLAND
MIR 168	10/08/95	Crinan Canal, Lochgilphead, Kintyre, Strathclyde, SCOTLAND
MIR 169	10/08/95	River Add, Bridgend, Kintyre, Strathclyde, SCOTLAND
MIR 170	10/08/95	Loch Coille-Bharr, Knapdale Forest, Kintyre, Strathclyde, SCOTLAND
MIR 171	10/08/95	Loch Eck, north of Donoor, Strathclyde, SCOTLAND
MIR 172	11/08/95	Loch Ascog, Isle of Bute, Strathclyde, SCOTLAND
MIR 173	11/08/95	River Leven, Renton, north of Dumbarton, Strathclyde, SCOTLAND
MIR 174	11/08/95	Carman Resevior, Renton, north of Dumbarton, Strathclyde, SCOTLAND
MIR 175	09/08/95	Loch on B846, below Rannoch, Tayside, SCOTLAND
MIR 176	21/09/95	Embalsa del Ebro, Canlabrica, SPAIN

CODE	DATE	SITE
MIR 177	22/09/95	Embalsa de Aguilar de Campo, Aguilar, SPAIN
MIR 178	22/09/95	Rio Rivero, Ruesaga, SPAIN
MIR 179	22/09/95	Rio Carrion, Velilla delCarrion, SPAIN
MIR 180	23/09/95	Rio Sil, Ponferrada, between Villa Patos and Toraldelosv, SPAIN
MIR 181	23/09/95	Rio Sil, Ponferrada, below Penarrubia dam and Salas de la Ribera, SPAIN
MIR 182	24/09/95	Lago de Sanabria, above Puebla Sanabria, SPAIN
MIR 183	24/09/95	Rio Tera, Puebla Sanabria, SPAIN
MIR 184	24/09/95	Rio Sabor, south of Rabal, PORTUGAL
MIR 185	24/09/95	Rio Igrejas, Gamonde, PORTUGAL
MIR 187	24/09/95	Rio Macas. Spanish Portugese border, PORTUGAL
MIR 188	25/09/95	Rio Coa, Vilar to Sabugal Road, PORTUGAL
MIR 189	25/09/95	Rio Zezere, Caria to Teixosa Road, PORTUGAL
MIR 190	25/09/95	Rio Dao, N231 north of Constancia, PORTUGAL
MIR 191	26/09/95	Rio Tejo, south of Constancia, PORTUGAL
MIR 192	18/10/95	Chester Canal, Chester, Cheshire, ENGLAND
MIR 193	17/10/95	Llyn, Clwyd, WALES

Appendix 2

Fungal species isolated from *Myriophyllum spicatum* during two years of surveying in Europe

Absidia cylindrospora Hagem.
Acremonium strictum W. Gams.
Acremonium persicinum (Nicot.) W. Gams.
Acrophialophora levis Samson & T. Mahmood.
Alternaria infectoria E.G. Simmons. Agg.
Apiospora montagnei Sacc.
Ascochyta sp. Lib.
Aureobasidium sp.Viola & Boyer.
*Byssochlamys nivea*Westling.
Botrytis cinerea Pers.
Chrysosporium sp. Corda
Cladobotryum sp.Corda
Colletotrichum dematium (Pers.: Fr.) Grove.
Coniothyrium fuckelii Sacc.
Coniothyrium sporulosum (W. Gams & Domsch) Aa.
Corynascus sepedonium (Emm.) Arx.
Cryptosporiopsis sp. Bub. & Kabat.
Cylindrocarpon destructans (Zinssm.) Scholten.
Cylindrocarpon aquaticum (Sv. Nilsson) Maranova & Descals
Cylindrocladium sp. Morgan
Embellisia sp. *Embellisia* cf. *telluster* E.G. Simmons.
Emericellopsis minima Stolk.
Fusarium acuminatum Ellis & Everhart
Fusarium avenaceum (Fr.) Sacc.
Fusarium crookwellense Burgess, P.E. Nelson & Touss.
Fusarium culmorum(W.G. Sm.) Sacc.
Fusarium equisitii (Corda) Sacc.
Fusarium flocciferum Corda.
Fusarium graminearum Schwabe.
Fusarium oxysporum Schlecht.
Fusarium poae (Peck) Wollenweber.
Fusarium sambucinum Fuckel
Fusarium solani (Martius) Sacc.
Fusarium sporotrichiodes Sherbak.
Fusarium sp. Link.
Geotrichum candidum Link.
Gliocladium catenulatum J.C. Gilman & E.V. Abbott.
Gliocladium roseum Banier.
Gliomastix murorum var. *felina* (Marchal) S. Hughes.
Glomerella cingulata (Stoneman) Spauld.& H. Schrenk.
Microdochium tabacinum (T.H. Beyma) Arx.
Microsphaeropsis sp. Höhn.
Mycocentrospora acerina (Hartig) Deighton.
Myrothecium cinctum (Corda) Sacc.
Myrothecium roridum Tode.
Nectria discophora (Mont.) Mont.
Nectria lugdunensis J.Webster
Phaeoseptoria sp. Speg.
Phoma complanata (Tode) Desm.
Phoma dennisii Boerema.
Phoma eupyrena Sacc.
Phoma exigua Desm.
Phoma hedericola (Dur. & Mont.) Boerema.

Phoma leveillei Boer. & G.J. Bollen.
Phoma macrostroma Mont.
Phoma nebulosa (Pers.:Fr.) Berk.
Phoma tropica R. Schneid.& Boerema.
Phoma sect. Paraphoma (Morgan-Jones & White) Boerema
Phoma sp. Desm.
Phomopsis sp.Sacc.
Pithomyces chartarum (Berk. & M.A. Curtis) M.B. Ellis
Plectosphaerella cucumerina (Lindf.) Gams.
Pythium sp. Pringsh.
Pythium sp. group F
Pythium sp. group HS
Pythium sp. group T
Pythium aquatile Höhnk.
Pythium acanthophoron Sideris.
Pythium periplocum Drechsler.
Pythium scleroteichum Drechsler.
Sclerotium hydrophilum Sacc.
Stagonospora sp. Sacc.
Saprolegnia parasitica Coker.
Trichosporiella sporotrichoides Oorschot.
Verticillium nigrescens Pethybr.

Appendix 3

Isolates which have been screened against sections of *Myriophyllum spicatum*

CODE	ISOLATE	RESULT
MIR 1	*Plectosphaerella cucumerina*	No Response
MIR 2	*Fusarium* sp.	No Response
MIR 2v	*Acremonium strictum*	No Response
MIR 2iii	*Gliocladium roseum*	No Response
MIR 2ia	*Pythium* sp.	No Response
MIR 3iii	*Embellisia* nr. *telluster*	good control
MIR 3a	*Embellisia* nr. *telluster* (reisolated 3iii)	good control
MIR 4vi	*Acremonium strictum*	No Response
MIR 4xa	*Fusarium sporotrichoides*	No Response
MIR 5v	*Fusarium crookwellense*	No Response
MIR 5i	*Apiospora montagnei*	No Response
MIR 5ix	*Acremonium strictum*	No Response
MIR 5e	*Byssochlamys nivea*	No Response
MIR 5va	*Fusarium crookwellense*	No Response
MIR 5iv	*Fusarium sporotrichoides*	No Response
MIR 5xv	*Fusarium sporotrichoides*	No Response
MIR 5iii	*Acremonium persicinum*	No Response
MIR 6	*Verticillium nigrescens*	No Response
MIR 6vi	*Acremonium strictum*	No Response
MIR 7a	*Auerobasidium* sp.	No Response
MIR 7xii	*Acremonium strictum*	No Response
MIR 7xiii	*Fusarium avenaceum*	No Response
MIR 8b	*Acremonium* sp.	No Response
MIR 13ii	*Fusarium sambucinum*	No Response
MIR 13i	*Fusarium sambucinum*	No Response
MIR 16ii	*Fusarium graminearum*	No Response
MIR 16ii	*Fusarium sambucinum*	No Response
MIR 16i	*Fusarium avenaceum*	No Response
MIR 16iii	*Fusarium culmorum*	No Response
MIR 16vii	*Fusarium culmorum*	No Response
MIR 16	*Fusarium solani*	good control
MIR 16b	*Fusarium oxysporum*	No Response
MIR 16a	*Fusarium acuminatum*	No Response
MIR 17j	*Acremonium strictum*	No Response
MIR 17	*Fusarium graminearum*	No Response
MIR 18	*Mucor hiemalis*	No Response
MIR 18	*Alternaria alternata*	No Response
MIR 18c	*Coniothyrium sporulosum*	No Response
MIR 18a	*Coniothyrium sporulosum*	No Response
MIR 22i	*Verticillium* sp.	No Response
MIR 22	*Fusarium polyphialides*	No Response
MIR 22r	*Fusarium oxysporum*	No Response
MIR 23	*Embellisia indefessa*	No Response
MIR 23	*Ascochyta* sp.	No Response
MIR 23	*Fusarium crookwellense*	No Response
MIR 24	*Cylindrocladium* sp.	No Response
MIR 24i	*Mucor hiemalis*	No Response
MIR 25	*Cylindrocladium* sp.	No Response
MIR 25ii	*Gliocladium roseum*	No Response
MIR 25iii	*Gliocladium roseum*	No Response
MIR 25i	*Gliocladium roseum*	No Response
MIR 25iv	*Gliocladium roseum*	No Response

CODE	ISOLATE	RESULT
MIR 26	Oomycete	No Response
MIR 26a	*Fusarium graminearum*	No Response
MIR 26	*Acremonium* sp.	No Response
MIR 27e	*Gliocladium roseum*	No Response
MIR 27d	*Gliocladium roseum*	No Response
MIR 27f	*Acremonium* sp.	No Response
MIR 27ii	*Trichosporiella sporotrichoides*	No Response
MIR 27g	*Pythium aquatile*	No Response
MIR 27i	*Epicoccum nigrum*	No Response
MIR 28	*Pythium sclerotoichum*	No Response
MIR 29b	*Acremonium* sp.	No Response
MIR 29c	*Cylindrocarpon* sp.	No Response
MIR 30	*Phomopsis* sp.	No Response
MIR 30b	*Cylindrocarpon* sp.	No Response
MIR 30a	*Cylindrocladium* sp.	No Response
MIR 30	*Phoma* sp.	No Response
MIR 30i	*Stagonospora* sp.	No Response
MIR 30	*Pythium* sp.	No Response
MIR 31a	*Fusarium oxysporum*	No Response
MIR 32	*Phoma* sp.	No Response
MIR 32d	Indeterminate Hyphomycete	No Response
MIR 32	*Acremonium* sp.	No Response
MIR 32a	*Cylindrocarpon* sp.	No Response
MIR 32b	*Verticillium* sp.	No Response
MIR 32c	*Fusarium culmorum*	No Response
MIR 34	*Gliocladium roseum*	good control
MIR 34a	*Fusarium* sp.	No Response
MIR 34b	*Corynascus sepedonium*	No Response
MIR 35	*Fusarium sambucinum*	No Response
MIR 35	*Fusarium graminearum*	No Response
MIR 35	Indeterminate Coelomycete	good control
MIR 35a	Oomycete	No Response
MIR 36e	Indeterminate Ascomycete	No Response
MIR 36	*Gliocladium roseum*	No Response
MIR 36b	*Mortierella* sp.	No Response
MIR 36	*Gliomastic murorum* var. *felina*	No Response
MIR 36ii	Indeterminate Hyphomycete	No Response
MIR 37b	*Phomopsis* sp.	No Response
MIR 37d	*Acremonium* sp.	No Response
MIR 38b	*Phomopsis* sp.	No Response
MIR 38	*Acremonium* sp.	No Response
MIR 38a	*Absidia cylindrospora*	No Response
MIR 40a	*Acremonium* sp.	No Response
MIR 42	*Cylindrocladium* sp.	No Response
MIR 42b	*Fusarium sambucinum*	No Response
MIR 42	*Fusarium sambucinum*	No Response
MIR 42	*Cladobotryum* sp.	No Response
MIR 43a	*Fusarium sambucinum*	No Response
MIR 43	*Fusarium sambucinum*	No Response
MIR 43c	*Fusarium pallidoroseum*	No Response
MIR 43e	*Emericellopsis minima*	No Response
MIR 43f	*Acremonium* sp.	No Response
MIR 43	*Acremonium* sp.	No Response
MIR 43	*Phoma exigua*	No Response
MIR 43	*Phomopsis* sp.	No Response
MIR 43	*Fusarium sambucinum*	No Response

CODE	ISOLATE	RESULT
MIR 44	Indeterminate Hyphomycete	No Response
MIR 44	*Saprolegnia parasitica*	No Response
MIR 44a	*Cylindrocladium* sp.	No Response
MIR 44	Oomycete	No Response
MIR 45e	Oomycete	No Response
MIR 45f	*Cylindrocarpon destructans*	No Response
MIR 45a	*Cylindrocarpon destructans*	No Response
MIR 45c	*Cylindrocarpon destructans*	No Response
MIR 45b	*Acremonium* sp.	No Response
MIR 45	*Acremonium* sp.	No Response
MIR 45d	Oomycete	No Response
MIR 45h	Indeterminate Hyphomycete	No Response
MIR 47a	*Cladobotryum* sp.	No Response
MIR 48	Oomycete	No Response
MIR 49	*Cylindrocarpon* sp.	No Response
MIR 49a	Indeterminate Hyphomycete (reisolated MIR 49d)	good control
MIR 49b	*Gliocladium roseum*	No Response
MIR 49g	*Acremonium* sp.	No Response
MIR 49d	Indeterminate Hyphomycete	good control
MIR 50	*Acremonium* sp.	No Response
MIR 50	*Acremonium* sp.	No Response
MIR 51	*Glomerella cingulata*	good control
MIR 58	Oomycete	No Response
MIR 59	*Chrysosporium* sp.	No Response
MIR 59d	*Geotrichum candidum* (reisolated 59e)	good control
MIR 59e	*Geotrichum candidum*	not retested
MIR 59	*Geotrichum candidum* (reisolated 59c)	good control
MIR 59c	*Geotrichum candidum*	not retested
MIR 59g	*Chrysosporium* sp.	No Response
MIR 59	Indeterminate Hyphomycete	No Response
MIR 60a	*Fusarium equisiti*	No Response
MIR 64b	*Cylindrocladium* sp.	No Response
MIR 64c	*Coniothyrium fuckelii* (reisolated 64d)	good control
MIR 64d	*Coniothyrium fuckelii*	not retested
MIR 65a	*Gliocladium roseum* (reisolated 65b)	slight effect
MIR 65b	*Gliocladium roseum*	not retested
MIR 67a	*Pythium periplocum*	slight effect
MIR 67c	*Verticillium* sp.	No Response
MIR 68a	*Fusarium sambucinum*	No Response
MIR 68g	*Gliocladium roseum*	No Response
MIR 68h	*Gliocladium roseum*	No Response
MIR 68c	*Acremonium* sp.	good control
MIR 68a	*Fusarium sambucinum*	No Response
MIR 69a	Indeterminate Hyphomycete	No Response
MIR 70c	*Acremonium* sp.	No Response
MIR 70a	*Acremonium* sp.	No Response
MIR 71a	*Phomopsis* sp.	No Response
MIR 73c	*Fusarium avenaceum*	No Response
MIR 75b	Indeterminate Hyphomycete	No Response
MIR 75a	*Cylindrocladium* sp.	No Response
MIR 76a	*Acremonium* sp.	No Response
MIR 77a	*Fusarium coeruleum*	No Response
MIR 78b	*Cylindrocladium* sp. (reisolated 78g)	No Response
MIR 78g	*Cylindrocladium* sp.	No Response
MIR 78a	*Fusarium ciliatum*	No Response
MIR 79a	*Fusarium sambucinum*	No Response

CODE	ISOLATE	RESULT
MIR 80a	*Fusarium graminearum*	No Response
MIR 80b	*Cylindrocarpon destructans*	good control
MIR 80c	Indeterminate Hyphomycete	good control
MIR 80j1	Indeterminate Coelomycete	No Response
MIR 83a	*Acremonium* sp.	No Response
MIR 84a	*Mycocentrospora acerina*	No Response
MIR 85a	*Acremonium* sp.	No Response
MIR 86b	*Fusarium* sp.	No Response
MIR 86a	*Lemonniera* sp.	No Response
MIR 87c	*Leptosphaerulina* sp.	No Response
MIR 87b	*Fusarium sporotrichoides*	No Response
MIR 89b	*Acremonium* sp.	No Response
MIR 89e	*Fusarium* sp.	No Response
MIR 91b	*Gliocladium* sp.	No Response
MIR 92a	*Fusarium avenaceum*	No Response
MIR 93c	*Gliocladium roseum*	No Response
MIR 93b	*Gliocladium roseum* (reisolated 93g)	good control
MIR 93g	*Gliocladium roseum*	not retested
MIR 93e	*Acremonium* sp.	No Response
MIR 94d	Indeterminate Coelomycete	No Response
MIR 96b	*Fusarium* sp.	No Response
MIR 97c	*Colletotrichum* sp.	No Response
MIR 100a	*Fusarium culmorum*	No Response
MIR 101a	*Fusarium culmorum*	No Response
MIR 102a	*Fusarium* sp.	No Response
MIR 102c	*Fusarium equisiti*	No Response
MIR 102j2	*Cylindrocladium* sp.	No Response
MIR 103c	New Hyphomycete	No Response
MIR 104a	*Macrophoma* sp.	No Response
MIR 108a	*Phaeoseptoria* sp.	No Response
MIR 113a	*Coniothyrium* sp.	No Response
MIR 114a	*Phoma* sp.	No Response
MIR 115b	*Phaeoseptoria* sp.	No Response
MIR 115a	*Ascochyta* sp.	No Response
MIR 116a	*Coniothyrium* sp.	No Response
MIR 117a	*Coniothyrium* sp.	No Response
MIR 119a	Indeterminate Hyphomycete *	No Response
MIR 119j1	*Fusarium acuminatum*	No Response
MIR 120	Indeterminate Hyphomycete *	No Response
MIR 122a	*Acremonium* sp.	No Response
MIR 123b	*Acremonium* sp.	No Response
MIR 124b	Indeterminate Hyphomycete	No Response
MIR 124a	*Phoma* sp.	No Response
MIR 125b	*Acremonium* sp.	No Response
MIR 125a	*Cladosporium chladosporioides*	No Response
MIR 126a	*Phomopsis* sp.	No Response
MIR 126b	*Phoma* sp.	No Response
MIR 126c	*Acremonium* sp.	No Response
MIR 126d	*Fusarium equisiti*	No Response
MIR 127b	*Acremonium* sp.	No Response
MIR 128a	*Acremonium* sp.	No Response
MIR 128b	*Fusarium* sp.	No Response
MIR 129c	*Fusarium* sp.	No Response
MIR 129d	*Fusarium* sp.	No Response
MIR 129j1	Sclerotial Isolate	No Response
MIR 131b	*Fusarium* sp.	No Response

CODE	ISOLATE	RESULT
MIR 131a	Oomycete	No Response
MIR 132a	*Pythium acanthophoron*	No Response
MIR 133a	*Acrophialophora levis*	No Response
MIR 134a	*Cryptosporiopsis* sp.	good control
MIR 134c	*Coniothyrium* sp.	No Response
MIR 135j1	*Coniothyrium* sp.	No Response
MIR 136a	*Phoma* sect. *paraphoma*	No Response
MIR 138b	Indeterminate Hyphomycete	No Response
MIR 139c	*Mucor* sp.	No Response
MIR 139a	*Alternaria* sp.	No Response
MIR 139b	*Myrothecium roridum*	No Response
MIR 140b	*Fusarium sambucinum*	No Response
MIR 140a	*Phoma* sp.	No Response
MIR 140j1	*Gliocladium* sp.	No Response
MIR 141c	*Fusarium* sp.	No Response
MIR 141a	*Phoma* sp.	No Response
MIR 142a	*Pithomyces chartarum*	No Response
MIR 142b	*Acremonium* sp.	No Response
MIR 143c	*Fusarium* sp.	No Response
MIR 143a	*Fusarium* sp.	No Response
MIR 144b	Indeterminate Hyphomycete	No Response
MIR 144b	*Acremonium* sp.	No Response
MIR 144j1	*Alternaria* sp.	No Response
MIR 144j2	*Fusarium culmorum*	No Response
MIR 145c	*Fusarium* sp.	No Response
MIR 145a	*Coniothyrium* sp.	No Response
MIR 147a	*Alternaria* sp.	No Response
MIR 147c	*Myrothecium* sp.	No Response
MIR 148a	Indeterminate Coelomycete	No Response
MIR 148b	*Ascochyta* sp.	No Response
MIR 148c	*Fusarium* sp.	No Response
MIR 149a	*Sclerotium hydrophilum*	No Response
MIR 149b	*Sclerotium hydrophilum*	No Response
MIR 150a	*Ascochyta* sp.	No Response
MIR 151a	*Ascochyta* sp.	No Response
MIR 152a	*Fusarium* sp.	No Response
MIR 156a	*Alternaria* sp.	No Response
MIR 157a	*Fusarium graminearum*	No Response
MIR 158e	*Acremonium* sp.	No Response
MIR 158a	*Ascochyta* sp.	No Response
MIR 158b	*Ascochyta* sp.	No Response
MIR 158c	*Ascochyta* sp.	No Response
MIR 158d	*Ascochyta* sp.	No Response
MIR 159c	*Cylindrocladium* sp.	No Response
MIR 159a	*Cylindrocladium* sp.	No Response
MIR 159b	*Phoma* sp.	No Response
MIR 160a	*Acremonium* sp.	No Response
MIR 161a	Indeterminate Hyphomycete	No Response
MIR 161b	*Alternaria* sp.	No Response
MIR 162a	Oomycete	No Response
MIR 163a	Oomycete	No Response
MIR 163b	Oomycete	No Response
MIR 163c	Indeterminate Hyphomycete	No Response
MIR 164j1	*Ascochyta* sp.	No Response
MIR 164j2	*Phaeostalagmus* sp.	No Response
MIR 164a	Indeterminate Hyphomycete	No Response

CODE	ISOLATE	RESULT
MIR 166j2	*Phoma tropica*	No Response
MIR 166j3	*Phoma* sect. *paraphoma*	No Response
MIR 166j1	*Phoma dennisii*	No Response
MIR 167j2	*Phoma* sp.	No Response
MIR 168j2	*Nectria lugdunensis*	No Response
MIR 168j1	*Cylindrocarpon aquaticum*	No Response
MIR 169j1	Indeterminate Coelomycete	No Response
MIR 169j2	Indeterminate Coelomycete	No Response
MIR 169j3	Indeterminate Coelomycete	No Response
MIR 170j1	Indeterminate Coelomycete	No Response
MIR 171j1	*Phoma leveillei*	No Response
MIR 172j1	*Phoma hedericola*	No Response
MIR 173j1	*Phomopsis* sp.	No Response
MIR 174j1	*Coniothyrium* sp.	No Response